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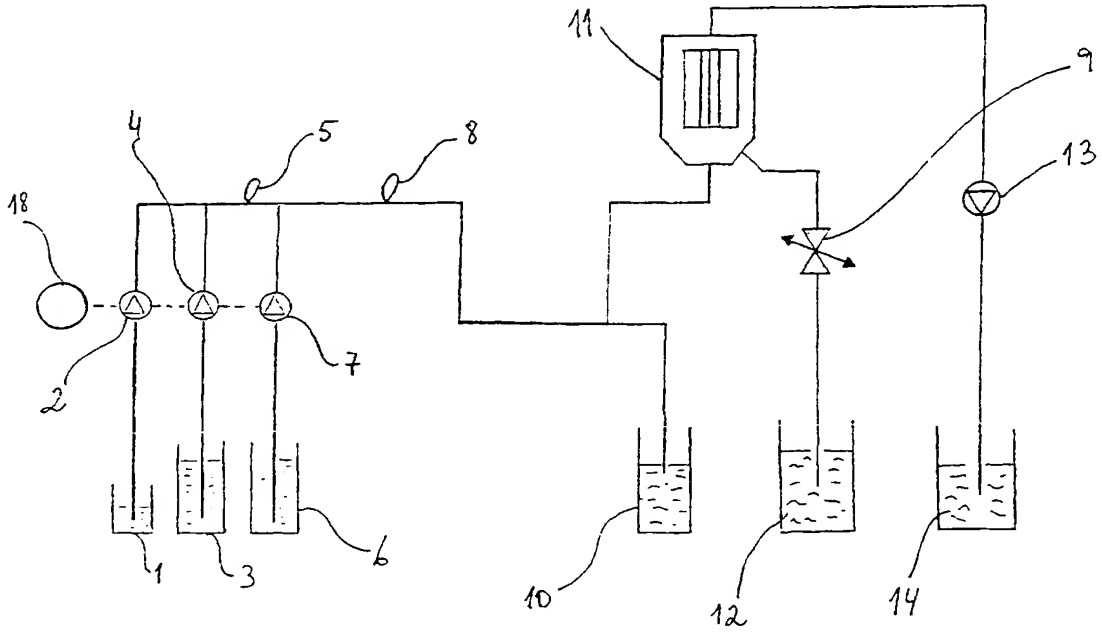
(54) Title: METHOD AND DEVICE FOR COUNTING CELLS IN URINE



(57) Abstract: The invention regards a method and a device for measuring the number of cells in urine. A fixative, a buffer and a dye are added to the urine sample, which is then analysed in a device for measuring fluorescence.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00286

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/493, G01N 33/50
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5693484 A (HIROYUKI NAKAMOTO ET AL), 2 December 1997 (02.12.97)	1-7
A	EP 0634640 A1 (OMRON CORPORATION), 18 January 1995 (18.01.95), page 5, line 26	4
A	WO 9403103 A1 (GUIRGUIS, RAOUF, A.), 17 February 1994 (17.02.94), page 3, line 32 - page 4, line 35, page 19, lines 15-27, page 20, line 15	1-7

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5545535 A (BRUCE L. ROTH ET AL), 13 August 1996 (13.08.96), column 1, lines 19-26, column 5, line 17; column 10, table 1; column 11, table 2	1-3,5-6

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INTERNATIONAL SEARCH REPORT
Information on patent family members

02/11/00

International application No.
PCT/NO 00/00286

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5693484 A	02/12/97	AU 1622692 A	19/11/92
		CA 2068471 A	15/11/92
		DE 69213315 D,T	23/01/97
		EP 0513762 A,B	19/11/92
		JP 3070968 B	31/07/00
		JP 4337459 A	25/11/92
EP 0634640 A1	18/01/95	JP 7333220 A	22/12/95
		US 5563070 A	08/10/96
WO 9403103 A1	17/02/94	AU 4777693 A	03/03/94
		BR 9306818 A	08/12/98
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		EP 0654972 A	31/05/95
		JP 8508395 T	10/09/96
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US 5545535 A	13/08/96	AU 676317 B	06/03/97
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		US 5658751 A	19/08/97
		WO 9424213 A	27/10/94
		JP 7196930 A	01/08/95

Method and device for counting cells in urine

The present invention regards a method and a device for counting bacteria and other micro-organisms in urine from a patient. The method and the device are very quick and accurate in terms of diagnosing cystitis. The technical area of the invention is medical diagnostics. The techniques used are covered by the areas of biochemistry/microbiology, optics, fluid mechanics, electronics and computer science. The novel aspects of the invention fall mainly within the subjects of biochemistry and microbiology.

The invention involves detecting bacteria by means of light scatter and fluorescence with an improved signal-to-noise ratio when compared with prior art.

Persons suffering from cystitis have cells in their urine that should not normally be there. These cells in the urine may be bacteria and fungus, as well as the patient's own cells (somatic cells), such as leukocytes or epithelial cells.

The invention seeks, through the method and device thereof, to solve the following problem: In the case of cystitis, it takes a long time (one or more days) to count the number of bacteria in urine. This because the bacteria must be cultivated on agar discs until they form macroscopic colonies than can be seen with the naked eye. The long wait required before a diagnosis can be made is unfortunate, as the patient is often given antibiotics before a certain diagnosis has been made.

Attempts have been made to solve this problem by counting the bacteria and other cells (lymphocytes and epithelial cells) directly in the urine by using specially designed cell counting devices (flow cytometers). In order to be able to do this, the cells must be made fluorescent by adding special fluorochromes that attach to the cells. In the flow cytometer, the cells are illuminated by a beam of light as they pass the measurement point in a liquid stream (thus flow cytometry). The instrument registers the light scatter and fluorescence from each individual cell. The intensity of the scattered light is a function of among other things the size of the cell, and the intensity of the fluorescent light is a function of among other things the amount of substance made fluorescent (e.g.

nucleic acids). The concentration of cells (number of cells per ml urine) is simply determined by counting the number of fluorescent particles in the sample. This may be grouped into different types of cells based on the size of the cells (light scatter) and the content of nucleic acids (fluorescence). There are also other quick methods of measuring bacteria in urine, however these are indirect and measure the presence of cellular metabolites (dipsticks).

The main problem associated with prior art that makes use of plate counting is the time it takes. The problem with today's flow cytometers is that they are not good enough at routinely measuring bacteria in urine, which are small in comparison with somatic cells (lymphocytes, epithelial cells).

US 5 693 484 regards a method of counting and classifying cells in urine. A fluorescent dye is added to the urine sample, which dye attaches to the nucleic acids of the cells. The cells are then illuminated with light at the blue and violet wavelengths, and analysed in a flow cytometer.

The method according to US 5 639 484 functions satisfactorily with somatic cells, but does not work well with bacteria. This is, among other things, due to the following facts:

- Using violet/blue excitation light results in auto-fluorescence, which causes the signal-to-noise ratio to be reduced at low fluorescence intensities (as in the case of bacteria).
- It is more difficult for live bacteria to absorb dye than it is for somatic cells, for several reasons.
- Firstly, the cell walls of the bacteria act as a barrier against the surroundings.
- Secondly, the bacteria may have intracellular pumps that bring the dye out again.
- Thirdly, the bacteria are considerably smaller than somatic cells, thus containing less of the cellular components that are to be stained.
- As a result of this, the fluorescence intensity per cell is low.

The present invention provides a method and a device that are reliable and quicker than the known techniques. The method consists of the following steps:

1. The urine sample from the patient is undiluted and is mixed with a fixative liquid so as to kill all the cells. The fixative liquids that may be used must be such that they render the cellular membrane permeable for absorption of the dyes (fluorochromes) mentioned below. Fixatives that may be used include ethanol, isopropanol and acetone, acetone being particularly preferred.

2. The mixture from point 1 has a buffer solution added to it, which is formulated so as to promote attachment of fluorochrome to the nucleic acids of the cells (DNA/RNA) (see point 3). At the same time, the buffer solution must prevent attachment to other cellular components. The buffer that has been found to be the most optimal is the so-called TBE-buffer (90 mM Tris, 90 mM Borate, 2,5 mM EDTA, pH 8).

3. A fluorochrome is added to the mixture from point 2, which fluorochrome specifically attaches to the nucleic acids of the cells. The present method may for instance involve the use of a monomer cyanine fluorochrome.

4. The mixture from point 3 is analysed in a device that measures light scatter and fluorescence from individual cells (e.g. a flow cytometer). The excitation light has a wavelength (635 nm) such that auto-fluorescence from the cells is insignificant.

5. The results are presented on a display that shows the fluorescent particles (cells) appearing separately (different colour) from particles without fluorescence, while displaying the absolute count. Cells in the lower size range (0.5 - 2µm) are assumed to be bacteria.

6. Steps 1 – 5 can be performed by a novel device according to the invention, such as appears in the accompanying schematic figure.

More specifically, the invention regards a method for counting cells in a urine sample, characterised in that a fixative is added to and mixed with the urine sample; a buffer solution is added to the mixture; followed by a dye; the mixture is then analysed in a device that measures light scatter and fluorescence from individual cells; and the results are shown directly on a display.

The invention further regards a device for measuring cells in a liquid stream by means of flow cytometry, in particular bacteria in a urine sample, characterised in that it comprises pickup tubes for the urine sample, which tubes lead to one or more mixing chambers to which are also connected separate receptacles for the fixative and the staining solution that are added to the mixing chamber via adjustable multi-channel pumps; the mixing chamber is further connected to an optical flow cytometric cell that receives carrier liquid from a receptacle.

According to the method of the invention, fluorescence is achieved by staining the bacteria. The cellular membrane is broken down when the cell is fixed by a fixative liquid such as ethanol, isopropanol or preferably acetone. The fixation also inactivates any eflux-pumps that may otherwise pump the dye back out of the cells. In this manner, the fluorochrome gains easy access to the intracellular components of the cells.

A further advantage is the fact that the method prevents auto-fluorescence by use of a dye that attaches specifically to nucleic acids and which is excited at light >500 nm (specifically 636 nm). The gain in fluorescence increases >10x upon attachment to the nucleic acids.

The method promotes specific attachment and reduces non-specific attachment by utilising special buffers, and the use of Tris-borate-EDTA, pH 8 has proven to be especially advantageous.

The device according to the invention, which may be used to implement the method, is explained schematically in greater detail in Figure 1.

The device consists of a connection for inlet of urine from a sampling bottle 1. The urine sample is sucked in by pump 2, and the sample is passed on to a mixing chamber or a reagent loop 5. A fixative such as ethanol or acetone is introduced into the mixing chamber 5 by pump 4. The staining solution is kept in receptacle 6 and is led to mixing chamber/reagent loop 8 by pump 7. A common motor 18 can drive pumps 2, 4, 7.

After the mixing has been completed in chamber 8, biological and chemical waste is separated out in a separate receptacle 10. The mixture of the urine sample, the fixative and the staining solution is sent on to the flow cell 11, in which the optical detection takes place. Light scatter is detected using MICROCYTE (Norwegian, European, US patent, pending Japan). For detection in the flow cell, use is made of a carrier liquid from receptacle 12. The amount and velocity of the carrier liquid 12 is adjusted by means of e.g. a throttle valve 9. Following detection of the sample in the flow cell 11, it is sent to waste container 14 by pump 13, which is connected to motor 17. This waste consists mainly of water with a very low content of biological material and chemicals.

The measurement of the urine sample in the flow cell is transferred to a data and control unit, where the results are shown on a display. The results are presented on a display where the fluorescent cells appear separately with a different colour from that of non-fluorescent particles. In addition, the total cell count is shown on the display. Cells in the lower size range from 0.5 to 2 μm are presented as bacteria.

The method and device according to the invention have a number of advantages over prior art, including the fact that they allow quicker and more reliable counting of bacteria in urine.

Using today's conventional plate technique, in which cultivated colonies of bacteria must be determined and counted using the naked eye, the analysis may take from one to several days, and may often require the sample to be sent away for analysis. By using the method and the device of the invention, the results of the analysis are available on site in a matter seconds.

A great advantage of the device is the fact that it is automated. There is no manual handling of chemicals, which removes the risk of the operator being exposed to any chemicals that may be injurious to his or her health.

The device also ensures a reduced possibility of human errors and failures during the handling and treatment of the sample.

By using the method and the device of the invention, the cost per sample will be lower than that which is the case for the conventional methods of analysis that are in use today.

C l a i m s

1.

A method for counting cells in a urine sample,

c h a r a c t e r i s e d i n that

- a fixative is added to and mixed with the urine sample;
- a buffer solution is added to the mixture, followed by a dye;
- the mixture is analysed in a device that measures light scatter and fluorescence from individual cells; and
- the results are shown directly on a display.

2.

A method according to Claim 1,

c h a r a c t e r i s e d i n that the fixative is of the type that renders the cellular membrane permeable, and may be acetone, ethanol or isopropanol, preferably acetone.

3.

A method according to Claim 1,

c h a r a c t e r i s e d i n that the buffer solution promotes attachment to the nucleic acids of the cells, and that it is preferably a TBE-buffer consisting of 90 mM Tris, 90 mM Borat, 2,5 mM EDTA, pH 8.

4.

A method according to Claims 1 – 2,

c h a r a c t e r i s e d i n that the dye used is a fluorochrome that specifically attaches to the nucleic acids of the cells, and that it is a monomer cyanine fluorochrome, preferably TOPRO-3.

5.

A method according to Claims 1 - 4,

c h a r a c t e r i s e d i n that the mixture is analysed in a device that measures light scatter and fluorescence from the individual cells, such as a flow cytometer.

6.

A method according to Claims 1 - 5,

c h a r a c t e r i s e d i n that the analyses are performed at a wave length >500, preferably at 635 nm.

7.

A device for measuring cells in a liquid stream by means of flow cytometry, particularly bacteria in a urine sample,

c h a r a c t e r i s e d i n that it comprises pickup tubes for a urine sample (1), which tubes lead to one or more mixing chambers (5, 8) to which are also connected separate receptacles for a fixative (3) and a staining solution (6) that are added to the mixing chamber (5, 8) via adjustable multi-channel pumps (2, 4, 7, 9), the mixing chamber further being connected to an optical flow cytometric cell (11) to which is added a carrier liquid from receptacle (12).

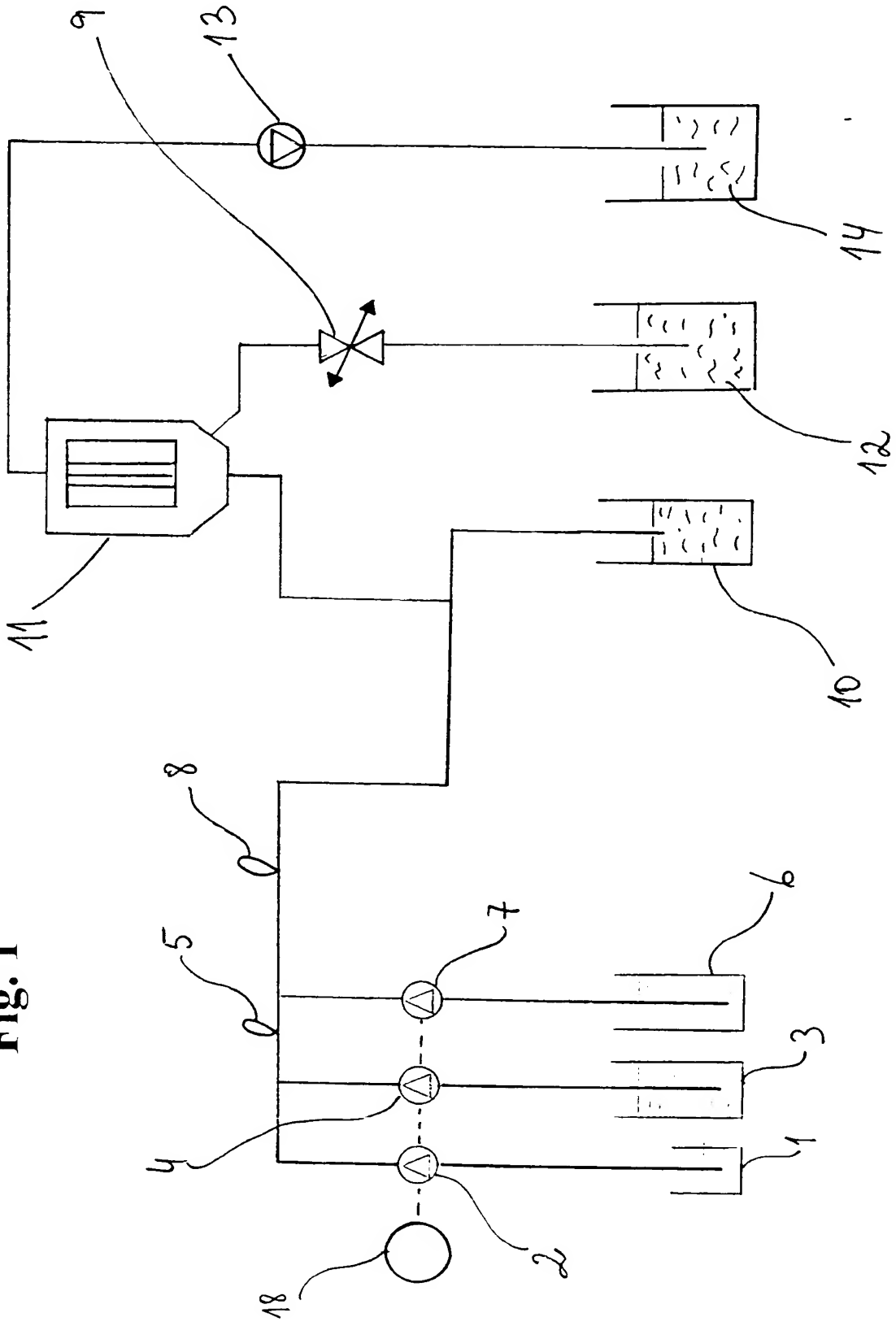

Fig. 1

PCT REQUEST

P1484PC00

Original (for **SUBMISSION**) - printed on 01.09.2000 03:51:53 PM

0 0-1	**For receiving Office use only** International Application No.	PCT/NO 00/00286
0-2	International Filing Date	01 SEPT. 2000 (01.09.00)
0-3	Name of receiving Office and "PCT International Application"	PATENTSTYRET Styret for det industrielle rettsvern ► PCT International application
0-4 0-4-1	**Form - PCT/RO/101 PCT Request** Prepared using	PCT-EASY Version 2.91 (updated 01.07.2000)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Norwegian Patent Office (RO/NO)
0-7	**Applicant's or agent's file reference**	P1484PC00
I	**Title of invention**	METHOD AND DEVICE FOR COUNTING CELLS IN URINE
II II-1 II-2 II-4 II-5 II-6 II-7 II-8 II-9	**Applicant** This person is: Applicant for Name Address: State of nationality State of residence Telephone No. Facsimile No.	applicant only all designated States except US OPTOFLOW AS P.O.Box 70 Bogerud N-0621 Oslo Norway NO NO +47-22627417 +47-22627275
III-1 III-1-1 III-1-2 III-1-4 III-1-5 III-1-6 III-1-7	**Applicant and/or inventor** This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence	applicant and inventor US only GJEISNES, Oddbjørn Gladvoll terrasse 2 N-1168 Oslo Norway NO NO

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III-2	**Applicant and/or inventor**	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	RØNNING, Øystein
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III-2-6	State of nationality	NO
III-2-7	State of residence	NO
IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name	PROTECTOR INTELLECTUAL PROPERTY CONSULTANTS AS
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IV-1-5	e-mail	mail@protectpat.no
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

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V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	01 September 1999 (01.09.1999)	
VI-1-2	Number	1999 4228	
VI-1-3	Country	NO	
VI-2	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1	
VII-1	**International Searching Authority Chosen**	Swedish Patent Office (ISA/SE)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	5	-
VIII-3	Claims	2	-
VIII-4	Abstract	1	abstractp1484pc00.txt
VIII-5	Drawings	1	-
VIII-7	TOTAL	13	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	1	
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IX-1	**Signature of applicant or agent**	Roger Mostue	
IX-1-1	Name	PROTECTOR INTELLECTUAL PROPERTY CONSULTANTS AS	
IX-1-2	Name of signatory	Roger Mostue	
IX-1-3	Capacity	Patent attorney	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	01 SEP 2000	(01.09.00)
10-2	**Drawings:**		
10-2-1	Received		
10-2-2	Not received	Received	

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10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/SE
10-6	Transmittal of search copy delayed until search fee is paid	X

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11-1	Date of receipt of the record copy by the International Bureau	25 SEPTEMBER 2000	(25.09.00)

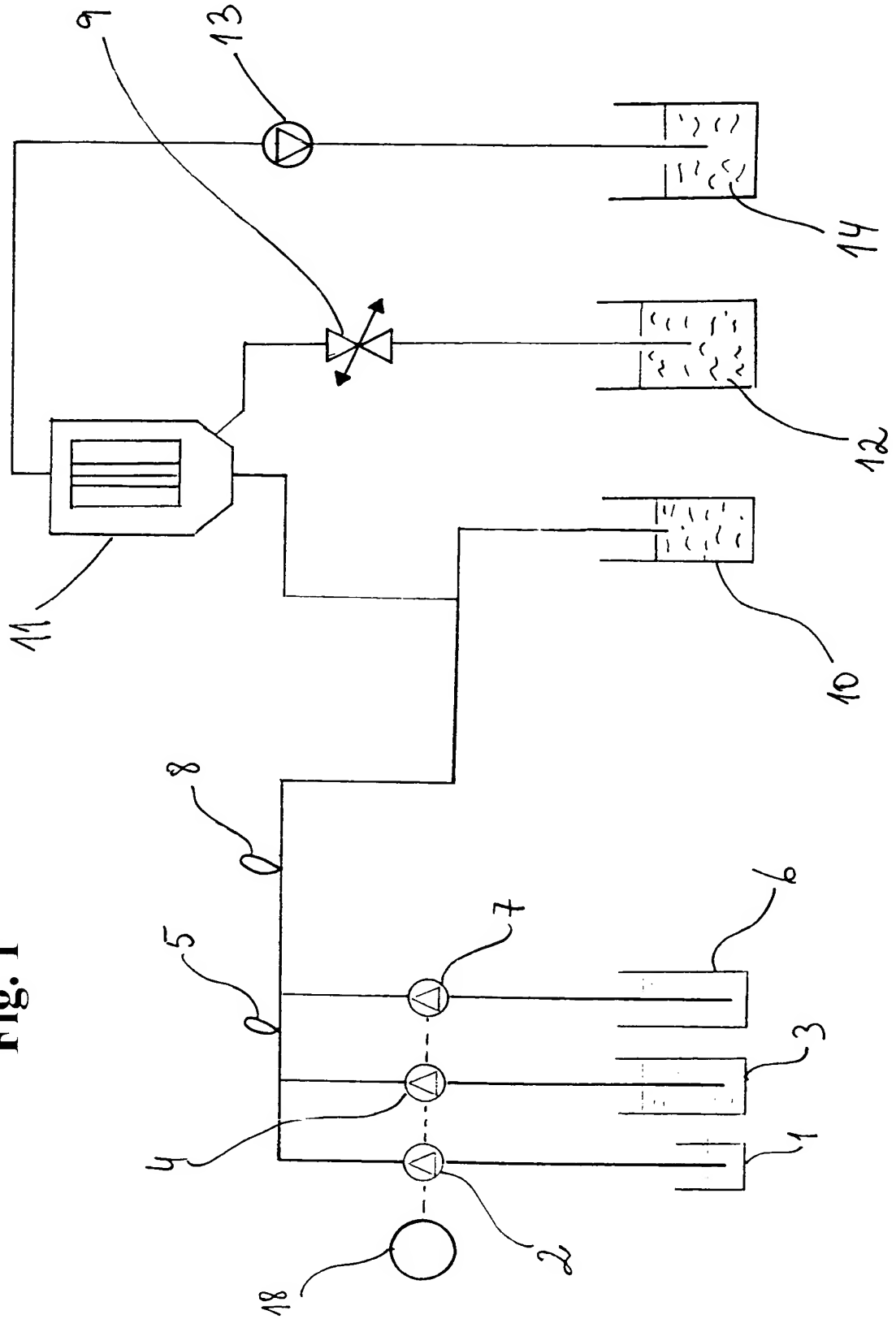

Fig. 1

ABSTRACT P1484PC00

The invention concerns a method and device for counting cells in urine. The urine sample is added a fixation agent, a buffer and a dye, and analyzed in a device measuring fluoroscense.

Metode og innretning for telling av celler i urin

Foreliggende oppfinnelse angår en metode og en innretning for å telle bakterier og andre mikroorganismer i urin fra en pasient. Metoden og innretningen er meget rask og nøyaktig med tanke på å diagnostisere urinveisinfeksjoner. Oppfinnelsens tekniske område er medisinsk diagnostikk. Teknikkene som benyttes omfattes av biokjemi/mikrobiologi, optikk, fluidmekanikk, elektronikk og datateknologi. Det nye ved oppfinnelsen er hovedsakelig innen fagområdene biokjemi og mikrobiologi.

Oppfinnelsen dreier seg om det å detektere bakterier ved hjelp av lysspredning og fluorescens med et bedre signal/støyforhold enn kjent teknikk.

Personer med urinveisinfeksjon har celler i urinen som normalt ikke skal være der. Disse cellene i urinen kan være bakterier og sopp, i tillegg til pasientens egne celler (somatiske celler), slik som leukocytter eller epitelceller.

Følgende problem søkes løst ved hjelp av metoden og innretningen i oppfinnelsen: Ved urinveisinfeksjoner tar det lang tid (en eller flere dager) å telle antall bakterier i urin. Dette fordi bakteriene må dyrkes på agarplater til de danner makroskopiske kolonier som kan telles med det blotte øye. Den lange ventetiden for å stille riktig diagnose er uheldig fordi pasienten ofte blir satt på antibiotikakur før sikker diagnose er stilt.

Dette problemet er søkt løst ved å telle bakteriene og andre celler (lymfocytter og epitelceller) direkte i urinen med spesialutviklede celletellingsapparater (flowcytometre). For å kunne gjøre dette må cellene gjøres fluorescerende ved at man tilsetter spesielle fluorochromer som binder seg til cellene. I flowcytometeret belyses cellene med en lysstråle i det de passerer målepunktet i en væskestrøm (derav flowcytometri). Instrumentet registrerer lysspredning og fluorescens fra hver enkelt celle. Intensiteten til det spredte lyset er en funksjon bl.a. av cellens størrelse, og intensiteten til det fluorescerende lyset er en funksjon bl.a. av mengden av den substans som gjøres fluorescerende (f eks nukleinsyrer). Konsentrasjonen av celler (antall celler per ml urin) kan enkelt telles ved å telle antall fluorescerende partikler i prøven. Dette kan grupperes i forskjellige celletyper utfra cellenes størrelse (lysspredning) og innhold

av nukleinsyrer (fluorescens). Det finnes også andre raske metoder som måler bakterier i urin, men disse er indirekte og måler tilstedeværelsen av cellulære metabolitter (dipsticks).

Hovedproblemet med kjent teknikk som benytter platetellinger er at det tar lang tid. Problemet med dagens flowcytometre er at de ikke er gode nok til rutinemessig å kunne måle bakterier i urin som er små i forhold til somatiske celler (lymfocytter, epitelceller).

US 5,693,484 angår en metode for telling og klassifisering av celler i urin. Urinprøven blir tilsatt et fluorescerende fargestoff som binder seg til cellenes nukleinsyrer. Cellene bestråles med lys ved blå og fiolett bølgelengde og analyseres i et flowcytometer.

Metoden i US 5,639,484 virker tilfredsstillende på somatiske celler, men problemet er at den virker dårlig på bakterier. Dette skyldes bl.a.:

- Ved eksitasjonslys i fiolett/blått får man autofluorescens som gjør at signal/ støyforholdet blir dårlig ved lave fluorescens intensiteter (som ved bakterier).
- Opptak av fargestoff i levende bakterier er vanskeligere enn i somatiske celler på grunn av flere forhold.
- For det første virker bakteriens cellevegg som en barriere mot omgivelsene.
- For det andre kan bakteriene ha intracellulære pumper som bringer fargestoffet ut igjen.
- For det tredje er bakterier adskillig mindre enn somatiske celler og inneholder dermed mindre av de cellulære komponentene som skal farges.
- Som følge av dette blir fluorescensintensiteten per celle derfor lav.

Foreliggende oppfinnelsen skaffer til veie en metode og innretning som er pålitelig og raskere enn de kjente teknikkene Metoden består av følgende trinn:

1. Urinprøven fra pasienten er ufortynnet og blandes med fikseringsvæske slik at alle cellene dør. Fikseringsvæskene som kan benyttes må ha den egenskap at de gjør cellemembranen permeabel for opptak av de nedenfor nevnte fargestoffer

(fluorochromer). De fikseringsvæskene som benyttes er for eksempel etanol, isopropanol, eller aceton, og blant disse er særlig aceton foretrukket.

2. Blandingen fra pkt 1 tilsettes en bufferløsning som er formulert slik at den fremmer binding av fluorochrom til cellenes nukleinsyrer (DNA/RNA) (se pkt 3). Bufferløsningen skal samtidig forhindre binding til andre cellulære bestandeler. Den buffer som er funnet å være mest optimal er såkalt TBE-buffer (90 mM Tris, 90 mM Borate, 2,5 mM EDTA, pH 8).

3. Blandingen fra pkt 2 tilsettes et fluorochrom som binder seg spesifikt til cellenes nukleinsyrer. I foreliggende metode brukes det f. eks. et monomert cyanin fluorochrom.

4. Blandingen fra pkt 3 analyseres i en innretning som måler lysspredning og fluorescens fra enkeltceller (f. eks. et flow cytometer). Eksitasjonslyset er av en slik bølgelengde (635 nm) at autofluorescens fra cellene er uten betydning.

5. Resultatene presenteres på et display der de fluorescerende partiklene (cellene) fremkommer adskilt (annen farge) fra partikler uten fluorescens, samtidig som det absolutte telletallet blir vist. Celler i det laveste størrelsesområdet (0,5-2μm) antas å være bakterier.

6. Trinn 1 – 5 kan utføres av en ny innretning i følge oppfinnelsen slik det fremgår av vedlagte skjematiske figur.

Mer spesifikt angår oppfinnelsen en metode for telling av celler i en urinprøve, kjennetegnet at urinprøven tilsettes og blandes med fikseringsvæske, blandingen tilsettes en bufferløsning, og deretter fargestoff, blandingen analyseres i en innretning som måler lysspredning og fluorescens fra enkeltceller, og resultatene blir vist direkte på et display.

Videre angår oppfinnelsen en innretning for måling av celler i en væskestrøm, ved hjelp av flowcytometri, særlig bakterier i en urinprøve, kjennetegnet ved at den omfatter opptaksrør for urinprøve som leder til et eller flere blandingskamre til hvilke det også er koblet separate beholdere for fikseringsvæske og fargeløsning som blir tilført blandingskammeret via regulerbare multikanals pumper, blandingskammeret er koblet videre til en optisk flowcytometrisk celle som blir tilført føringvæske fra beholder.

I følge metoden i oppfinnelsen blir fluorescens oppnådd ved at bakteriene blir farget. Cellemembranen blir brutt ned når cellen fikseres med fikseringsvæske som etanol, isopropanol eller fortrinnsvis aceton. Fikseringen inaktiverer også eventuelle efflux-pumper som ellers kan pumpe fargestoffet ut av cellene. På denne måten får fluorochromet lett adgang til cellens intracellulære komponenter.

En annen fordel er at metoden forhindrer autofluorescens ved å benytte fargestoff som bindes spesifikt til nukleinsyrer og som eksiteres ved lys >500 nm (spesifikt 636 nm). Fluorescensutbytte økes >10x ved binding til nukleinsyrene.

Metoden fremmer spesifikk binding og reduserer uspesifikk binding ved å benytte spesielle buffere, og særlig har det vist seg fordelaktig å benytte Tris-borate-EDTA, pH 8.

Innretningen i følge oppfinnelsen som kan benyttes til å gjennomføre fremgangsmåten blir forklart nærmere skjematisk i Figur 1.

Innretningen består av en forbindelse for inntak av urin fra et prøvetakingsglass 1. Urinprøven suges inn ved hjelp av pumpe 2 og prøven føres videre til et blandekammer eller reagensloop 5. Fikseringsvæske som for eks. etanol eller aceton blir tilført blandekammeret 5 ved hjelp av pumpe 4. Fargeløsningen oppbevares i beholder 6 og ledes til blandekammer/reagensloop 8 ved hjelp av pumpe 7. En felles motor 18 kan drive pumpene 2, 4, 7.

Etter at blandingen er gjennomført i kammer 8 blir biologisk og kjemisk avfall skilt ut i egen beholder 10. Blandingen av urinprøve, fikseringsvæske og fargeløsning føres

videre til flowcellen 11 der den optiske deteksjonen foregår. Lysspredning detekteres med MICROCYTE (norsk, europeisk US patent, pending Japan). Til deteksjon i flowcellen benyttes en føringsvæske fra beholder 12. Mengde og hastighet på føringsvæsken 12 blir regulert ved hjelp av f. eks. en strupeventil 9. Etter deteksjon av prøven i flowcellen 11 føres den videre til avfallsbeholder 14 ved hjelp av pumpe 13 tilkoblet motor 17. Dette avfallet består hovedsakelig av vann med meget lavt innhold av biologisk materiale og kjemikalier.

Målingen av urinprøven i flowcellen blir overført til en data- og kontrollenhet der resultatene blir vist på et display. Resultatene presenteres på et display der de fluorescerende cellene fremkommer adskilt med en annen farge enn partikler uten fluorescens. I tillegg blir det absolutte celletallet vist på displayet. Celler i det lavere størrelsesområde fra 0,5 til 2 μm blir presentert som bakterier.

Metoden og innretningen i følge oppfinnelsen har en rekke fordeler i forhold til den kjente teknikken blant annet ved at den er mye raskere og mer pålitelig for telling av bakterier i urin.

Med dagens konvensjonelle plateteknikk der dyrkede bakteriekolonier må bestemmes og telles med det blotte øyet, kan analysen ta fra én til flere dager og ofte må prøvene sendes vekk. Med metoden og innretningen i følge oppfinnelsen, får man analyseresultatene på stedet i løpet av noen sekunder.

En stor fordel med innretningen er at den er automatisert. Det forekommer ikke noen manuell bruk av kjemikalier, og som følge av dette blir operatøren ikke eksponert for eventuelle helsefarlige kjemikalier.

Innretningen sørger også for redusert mulighet for menneskelige feil og svikt under prøvebehandlingen.

Med metoden og innretningen i følge oppfinnelsen vil prisen per prøve være lavere enn de konvensjonell analysemetodene som benyttes i dag.

P a t e n t k r a v

1.

Metode for telling av celler i en urinprøve,

k a r a k t e r i s e r t v e d at

- urinprøven tilsettes og blandes med fikseringsvæske,
- blandingen tilsettes en bufferløsning, og deretter fargestoff,
- blandingen analyseres i en innretning som måler lysspredning og fluorescens fra enkeltceller, og
- resultatene blir vist direkte på et display.

2.

Metode i følge krav 1,

k a r a k t e r i s e r t v e d at fikseringsvæsken er av den typen som gjør cellemembranen permeabel, og kan være aceton, etanol eller isopropanol, fortrinnsvis aceton.

3.

Metode i følge krav 1,

k a r a k t e r i s e r t v e d at bufferløsningen fremmer binding til cellenes nukleinsyrer, og er fortrinnsvis en TBE-buffer som består av 90 mM Tris, 90 mM Borat, 2,5 mM EDTA, pH 8.

4.

Metode i følge krav 1 – 2,

k a r a k t e r i s e r t v e d at fargestoffet som benyttes er et fluorochrom som binder seg spesifikt til cellenes nukleinsyrer, er et monomert cyanin fluorochrom, fortrinnsvis TOPRO-3.

5.

Metode i følge krav 1 - 4,

k a r a k t e r i s e r t v e d at blandingen analyseres i en innretning som måler lysspredning og fluorescens fra enkeltceller, for eksempel i et flow cytometer.

6.

Metode i følge krav 1 – 5,

k a r a k t e r i s e r t v e d at analysene skjer ved en bølgelengde >500, fortrinnsvis ved 635 nm.

7.

Innretning for måling av celler i en væskestrøm, ved hjelp av flowcytometri, særlig bakterier i en urinprøve,

k a r a k t e r i s e r t v e d at den omfatter opptaksrør for urinprøve (1) som leder til et eller flere blandingskamre (5, 8) til hvilke det også er koblet separate beholdere for fikseringsvæske (3) og fargeløsning (6) som blir tilført blandingskammeret (5, 8) via regulerbare multikanals pumper (2, 4, 7, 9), blandingskammeret er koblet videre til en optisk flowcytometrisk celle (11) som blir tilført føringvæske fra beholder (12).